Evolution and classification of figs (Ficus, Moraceae) and their close relatives (Castilleae) united by involucral bracts

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Abstract

Figs and fig wasps are a classic example of an obligate pollination mutualism. Decades of work untangling the ecology and evolution of these organisms has simultaneously contributed to development of the fields of mutualism, coevolution and plant–insect interactions at large. With > 800 species, figs (*Ficus*, Moraceae) are among some of the larger genera of angiosperms. Phylogenetic studies of Moraceae have supported the clade Castilleae as the sister lineage of *Ficus*. Compared to *Ficus*, Castilleae have many fewer species (60 species in 11 genera), suggesting changes in rates of diversification along these two branches. Relatively little is known about Castilleae compared to *Ficus*, and we argue that defining the clade comprising *Ficus* and Castilleae, hereafter Involucrata, focuses attention on opportunities for comparative studies of pollination mutualisms and diversification rates. In this study, we define Involucrata and propose a revised classification scheme that accounts for the phylogenetic reconstruction based on the most

comprehensive sampling of this group to date. Moving forward, this classification will better guide and support evolutionary, ecological and comparative pollination biology studies of this group.

Additional Keyword: Asperae – external transcribed spacer – Involucrata – Mixtiflores – *Noyera* – paralogy – phylogenetic reconstruction.

Introduction

With at least 800 named species, *Ficus* L. accounts for more than half of the species diversity of the mulberry family, Moraceae (*c.* 1100 species; Clement & Weiblen, 2009). Phylogenetic analyses of Moraceae have strongly supported Castilleae as sister to *Ficus* based on plastid (Datwyler & Weiblen, 2004), nuclear (Zerega *et al.*, 2005) and morphological data (Clement & Weiblen, 2009). *Ficus* has been central to advancing study of pollination mutualisms, coevolution and cospeciation (Bronstein, 1988; Herre, 1989; Herre & West, 1997; Lopez-Vaamonde *et al.*, 2001; Weiblen, 2001; Weiblen, Yu & West, 2001; Weiblen & Bush, 2002; Cook & Rasplus, 2003; Jousselin, Rasplus & Kjellberg, 2003; Weiblen, 2004; Machado *et al.*, 2005; Rønsted *et al.*, 2005; Marussich & Machado, 2007; Silvieus, Clement & Weiblen, 2007; Jackson *et al.*, 2008; Jousselin *et al.*, 2008; Herre, Jandér & Machado, 2008; Cruaud *et al.*, 2012a; Cruaud *et al.*, 2012b; McLeish & van Noort, 2012; Conchou *et al.*, 2014; Bain *et al.*, 2016; Rodriguez *et al.*, 2017). *Ficus* spp. occur in tropical and subtropical regions worldwide and include trees, hemiepiphytes, epiphytes, shrubs, climbers, rheophytes and lithophytes. In contrast, Castilleae are a group of 11 genera and 60 species of trees and shrubs with four species distributed in the Palaeotropics and 56 species in the Neotropics. *Ficus* and Castilleae diverged from one another at least 65 Mya (Zerega *et al.*, 2005), and the striking difference in contemporary species richness suggests differing rates of diversification.

Together, *Ficus* and Castilleae differ from other Moraceae in having involucral bracts that subtend the inflorescences on a disc or urn-shaped receptacle. In Castilleae, the involucral bracts do not completely enclose the inflorescence like they do in *Ficus*. The positioning of these bracts has profound implications for their reproductive ecology. In *Ficus*, the involucral bracts form a tight pore, or ostiole, at the apex of the receptacle. Mated pollinating wasps force themselves through this opening into the cavity of the fig (syconium) where they pollinate flowers, lay eggs and usually die. Pollinator offspring emerge from galls inside the fig to mate and collect pollen from staminate flowers before exiting in search of other receptive figs. In contrast to the ‘cradle to grave’

relationship between figs and their pollinating wasps, Castilleae inflorescences are only partially enclosed by involucral bracts thereby allowing pollinators to come and go. From the limited study of pollination in Castilleae, wind (Osmaston, 1965; Croat, 1978) and insect (Sakai, Kato & Nagamasu, 2000; Zerega, Mound & Weiblen, 2004) pollination syndromes are present. As in *Ficus*, insect-pollinated Castilleae are also involved in broodsite pollination mutualisms in which pollinators mate and lay eggs in the inflorescences. Pollination by thrips has been documented for two species of Castilleae, *Antiaropsis decipiens* K.Schum. (endemic to New Guinea; Zerega *et al.*, 2004) and *Castilla elastica* Sess. (widespread in the Neotropics; Sakai *et al.*, 2000).

Comparative study of *Ficus* and Castilleae can offer insights into the evolution of morphological and molecular diversity, pollination ecology, diversification rates and historical dispersal patterns. However, aside from family-level phylogenetic studies (Datwyler & Weiblen, 2004; Zerega *et al.*, 2005; Clement & Weiblen, 2009), *Ficus* and Castilleae have seldom been the subject of comparative work (Clement, 2008; Moe, Clement & Weiblen, 2012). Comparing Castilleae and fig pollination syndromes, Moe *et al.* (2012) hypothesized that the nature of the pollinator reward and the number of floral visits by a pollinator may account for the difference in diversification in these two lineages. For instance, fig wasp offspring develop in galled or fertilized fig ovules. When wasp offspring fare better in pollinated flowers, pollination can increase wasp fitness and the fig can furthermore reduce pollen production to the benefit of pollinator production. Thrips pollinating Castilleae do not depend on successful pollination as thrips eat pollen and mate on male inflorescences. Selective pressure on host choice also differs among fig and Castilleae pollination syndromes. In many species, foundress fig wasps lose their wings and antennae on entering a fig so that they cannot reach another tree, probably resulting in intense selection to discern host quality before host selection. Castilleae pollinators can visit multiple inflorescences per generation with little consequence for visiting a non-rewarding inflorescence. Differing selective pressures resulting from the nature of these pollination interactions may have impacted the evolutionary trajectory of both lineages (Moe *et al.*, 2012). Further testing of this hypothesis requires additional study of pollination biology of Castilleae and an improved phylogenetic framework for *Ficus* and Castilleae.

Our current understanding of *Ficus* classification is largely based on a massive Malesian revision of *Ficus* initiated by Corner and completed by Berg after Corner's death (Berg, 2003a, b, c, d, e, 2004a, b; Berg & Corner, 2005) building on earlier work (summarized in Corner, 1965). Berg's

classification based on morphological and anatomical characters added emphasis on vegetative characters compared to Corner's treatments that focused on floral and fruit characters (Corner, 1965). Ultimately, Berg & Corner (2005) subdivided *Ficus* into six subgenera: (1) *Pharmacosycea* (Miq.) Miq. (monoecious); (2) *Urostigma* (Gasp.) Miq. (monoecious) (3) *Ficus* (gyno-dioecious); (4) *Sycidium* (Miq.) Mildbr. & Burret (gyno-dioecious); (5) *Synoecia* (Miq.) Miq. (gyno-dioecious) and (6) *Sycomorus* (Gasp.) Miq. (gynodioecious and monoecious). Subgenera *Pharmacosycea*, *Sycidium, Sycomorus* and *Urostigma* are distributed from the Pacific to West Africa, with subgenera *Pharmacosycea* and *Urostigma* additionally including a Neotropical section. Subgenera *Ficus* and *Synoecia* are almost exclusively restricted to the Malesian region and mainland Asia (Berg, 2003a).

The most recent comprehensive molecular phylogenetic analysis of 200 *Ficus* spp. supported the monophyly of subgenera *Sycidium, Sycomorus* and *Synoecia*, but subgenera *Ficus, Pharmacosycea* and *Urostigma* were paraphyletic (Cruaud *et al.*, 2012b) concurring with prior work on phylogenetic trees for *Ficus* (Weiblen, 2000; Jousselin *et al.*, 2003; Rønsted *et al.*, 2005; Rønsted *et al.*, 2008a; Xu *et al.*, 2011). Although many sections and subsections in these subgenera were not monophyletic, several supported clades do broadly correspond to published sections [*Adenosperma* Corner, *Americanae* Miq., *Eriosycea* Miq., *Galoglychia* Gasp., *Oreosycea* (Miq.) Miq., *Pharmacosycea* (Miq.) Benth.& Hook.f, *Sycocarpus* Miq., *Sycomorus* (Gasp.) Miq.] and subsections [*Conosycea* (Miq.) C.C.Berg, *Ficus*, *Frutescentiae* Sata, *Malvanthera* (Corner) C.C.Berg, *Urostigma* (Gasp.) C.C.Berg] (Berg & Corner, 2005; Rønsted *et al.*, 2008a). Given that phylogenetic evidence only partly supports previous taxonomic treatments based on morphology, there is much potential for confusion.

Relationships along the backbone of the phylogenetic tree for *Ficus* remain unsupported, and conflicts between ribosomal DNA and low-copy nuclear gene trees are not resolved (Cruaud *et al.*, 2012b; Harrison *et al.*, 2012). Further, a recent phylogenetic reconstruction from whole plastids representing 59 *Ficus* spp. (Bruun-Lund *et al.*, 2016) provided strong support for relationships deep in the phylogenetic tree for *Ficus*. However, a number of conflicts were identified and await increased resolution and clade support from phylogenetic trees reconstructed from nuclear gene regions for further investigation.

Similar to *Ficus*, the current classification of Castilleae is primarily based on morphology. Castilleae are trees, generally diagnosed by unisexual inflorescences with discoid to cup-shaped receptacles,

bracts subtending the inflorescence (involucre), large seeds, septate wood fibres and the lack of cystoliths. Molecular phylogenetic analysis of plastid (*ndhF*; Datwyler & Weiblen, 2004) and nuclear (*26S*; Zerega *et al.*, 2005) sequence data in addition to morphology (Clement & Weiblen, 2009) supported the unity of Castilleae, including *Antiaropsis* K.Schum, *Poulsenia*

Eggers and *Sparattosyce* Bureau (formerly part of tribe Artocarpeae, breadfruit and relatives) plus all eight genera of Neotropical Castilleae (Datwyler & Weiblen, 2004). Morphological analysis of the tribe further supported two subtribes, Antiaropsineae, comprising *Antiaropsis* and *Sparattosyce*, and Castillineae, including the remaining nine genera (Clement & Weiblen, 2009). As Castilleae have only been treated in the context of Moraceae, revision of classification of Castilleae awaits molecular phylogenetic study.

To facilitate further comparative work among *Ficus* and Castilleae, we present an improved phylogenetic framework for both clades. First, we propose the name Involucrata for the well-supported clade including Castilleae and *Ficus*. This name reflects a key morphological feature shared between the two lineages, involucral bracts. Next, we present a molecular phylogenetic tree of 307 *Ficus* spp. and 43 species of Castilleae, the most robust species sampling of the group to date. Finally, using the current classification of *Ficus* and Castilleae based on morphology (Berg, 1977; Berg & Corner, 2005; Berg, Corner & Jarrett, 2006), we use the phylogenetic tree reconstructed here as a framework to suggest revisions to the classification of Involucrata that now reflect evolutionary relationships. The clade Involucrata includes the reciprocally monophyletic tribes Castilleae and Ficeae.

Material and methods

Taxon sampling

To assess the current classification and describe the evolutionary relationships of *Ficus* and Castilleae, we assembled the most comprehensive data matrix to date, sampling representatives of all 11 genera of Castilleae and > 40% of 800 named *Ficus* spp. Data were assembled in two matrices. The first data matrix focused on phylogenetic reconstruction of Involucrata and included 133 taxa. Taxon sampling included 94 *Ficus* spp. (two or three species per major clade; Cruaud *et al.*, 2012b), 39 species of Castilleae representing all 11 genera, and *Artocarpus* J.R.Forst. & G.Forst.

(Artocarpeae, Moraceae) as an outgroup. This data set included three gene regions: the internal transcribed spacer region of nuclear ribosomal DNA (ITS), glyceraldehyde 3-phosphate dehydrogenase (*G3pdh*) and granule bound starch synthase (*GBSSI*; Supporting Information, Supplementary Table S1). The second matrix focused on *Ficus* and included 307 *Ficus* spp. adding > 100 species to the most recent comprehensive phylogenetic sample (Cruaud *et al.*, 2012b). Our sampling included the type species of traditionally recognised sections of *Ficus* wherever possible. We designated *Antiaropsis decipiens*, *Castilla elastica*, *Poulsenia armata* (Miq.) Standl. and *Sparattosyce dioica* Bureau as outgroups to root the phylogenetic tree. This data set included six gene regions: ITS; external transcribed spacer region (ETS) and four low-copy nuclear gene regions (*G3pdH*, *GBSSI*, glutamine synthase (*ncpGS*) and, for the first time for *Ficus*, Mg-protoporphyrim monomethyl ester cyclase (*At103*)) (Supporting Information, Supplementary Table S1).

Leaf material for sequencing newly added species was obtained from herbaria (A, AAU, F, HON, HUH, K, LAE, MIN, MO, PUH, UNAM), living collections (BG, BR, C, HITBC, K, NBG, REU) and recent field collections (Supporting Information, Supplementary Table S1). New data (> 400 = 34% of analysed sequences) were combined with data from prior phylogenetic work on Moraceae (Weiblen, 2000; Jousselin *et al.*, 2003; Machado *et al.*, 2005; Rønsted et al., 2005, 2008a, b; Silvieus *et al.*, 2007; Jackson *et al.*, 2008; Renoult *et al.*, 2009; Azuma *et al.*, 2010; Mcleish *et al.*, 2011; Xu *et al.*, 2011; Cruaud *et al.*, 2012b; Harrison *et al.*, 2012; Kusumi *et al.*, 2012; Chantarasuwan *et al.*, 2015). GenBank accessions for all taxa are available in Supporting Information, Supplementary Table S1.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from 15–30 mg of dried leaf-fragments or herbarium material following Rønsted *et al.* (2008a). Amplification of ITS, ETS, *G3pdh*, *ncpGS* and *GBSSI* for all *Ficus* spp. was performed following Cruaud *et al.* (2012b) and references therein. Amplification of At103 followed protocols by Li *et al.* (2008). Amplification primers are listed in Supporting Information, Supplementary Table S2.

ITS, *G3pdh* and *GBSSI* for genera of Castilleae were amplified in a 25 μL reaction using 1× *TaKaRa Ex Taq* buffer (2mM $MgCl_2$; Otsu, Shiga, Japan), 0.2 mM each dNTP, 10 μM bovine serum albumin (BSA), 12–25 μM forward and reverse primers (Supporting Information, Supplementary Table S2), 1.25 U *TaKaRa Ex Taq* DNA polymerase and *c*. 20 ng of genomic DNA. In instances when ITS

amplification was not successful, a nested PCR approach was used by first amplifying a larger region encompassing ITS with 25 μM of external primers 17SE and 26SE (Sun *et al.*, 1994), followed by a second PCR using 1 μL of the previous PCR product, and 25 μM of ITS4 and ITS5. Thermal cycler conditions for all ITS amplifications were: 94 °C for 2 min, 25 cycles of 94 °C for 1 min, 50 °C for 1 min, 70 °C for 2 min, followed by 72 °C for 7 min. Thermal cycler conditions for *G3pdh* were: 95 °C for 3 min 30 s, 35 cycles of 95 °C for 1 min, 49 °C for 1 min, 70 °C for 2 min, followed by 72 °C for 7 min. Thermal cycler conditions for *GBSSI* followed a 'stepdown' protocol modified from Evans *et al.* (2000) as follows: 94 °C for 3 min, 2 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min, 2 cycles of 94 °C for 1 min, 54 °C for 2 min, 72 °C for 2 min, 2 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min, and 24 cycles of 94 °C for 1 min, 48 °C for 2 min, 72 °C for 2 min, followed by 72 °C for 20 min. PCR products were column purified using a Qiagen PCR cleanup kit (Qiagen, Valencia, CA, USA) and quantified using a Turner Quantech Fluorometer (Barnstead-Thermolyne, Dubuque, IA, USA) using Hoecsht 33258 dye prior to sequencing.

All ITS, ETS, *G3pdh*, *ncpGS* and *At103* PCR products were directly sequenced. *GBSSI* and ITS amplicons showing signs of divergent alleles in direct sequencing were cloned prior to sequencing using either a TOPO-TA (Invitrogen, Carlsbad, CA, USA) or Stratagene PCR cloning kit (Agilent Technologies, Santa Clara, CA, USA) following manufacturer protocols. Transformed bacteria were grown overnight on LB + ampicillin agar plates at 37 °C. Eight to ten colonies per PCR product were screened using PCR for insert size. Three positive clones per accession were grown in LB + ampicillin broth overnight at 37 °C and plasmids were isolated using Qiagen Plasmid Isolation kit (Qiagen, Valencia, CA, USA). In other cases, the gene region of interest was cleaned directly from the clone screen PCR using a Qiagen PCR cleanup kit.

Previously published ETS trees for *Ficus* have been in conflict with other nuclear genes, as the ETS tree failed to recover a monophyletic *Ficus* subgenus *Sycomorus* because section *Sycocarpus* formed a separate clade sister to subgenus *Urostigma* (excluding subsection *Urostigma*) (e.g. Rønsted *et al.*, 2008a). Multiple copies of ETS in *Ficus* have been suspected (Cruaud, pers. comm.; NR pers. obs.) and potential problems with ETS paralogy have been reported (Calonje *et al.*, 2009). We explored the problem in *Ficus* by resampling species from clades in conflict and not in conflict among the ETS and other trees. Our sampling included: section *Sycocarpus* (*F. condensa* King, *F. fistulosa* Reinw. ex Blume, *F. hispida* Blanco and *F. scortechinii* King), section *Adenosperma* (*F.*

ochrochlora Ridl., *F. pseudopalma* Blanco and *F. itoana* Diels), and section *Sycomorus* (*F. sur* Forssk., *F. sycomorus* L. and *F. vallis-choudae* Delile) covering subgenus *Sycomorus*, subsection *Conosycea* (*F. drupacea* Thunb.) and subsection *Urostigma* (*F. lacor* Buch.-Ham). In an effort to capture a greater proportion of ETS paralogues potentially present, we relaxed PCR conditions by lowering the annealing temperature from 49 to 45 °C, increasing the number of cycles from 25 to 40, and extending the duration of the premelt from 2 min 30 s to 4 min. We also designed and used a *Ficus* specific primer (ETS-Fic1, Supporting Information, Supplementary Table S2), and cloned all PCR products. We column purified and sequenced six to nine clones per accession (except for *F. hispida* in which only three amplicons were recovered).

Sequencing for all cleaned PCR products was performed using Big Dye v.3.1 sequencing reagents and protocols (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were performed in 10 μL reactions with 20 ng PCR product or 200 ng of isolated plasmids. Sequencing primers for each gene region are listed in Supporting Information, Supplementary Table S2. Products were visualized and data were collected on an ABI 377 automated DNA sequencer (Applied Biosystems). Sequences were assembled using Sequencher v.4.6 (Gene Codes Corp., Ann Arbor, MI, USA) or Geneious v.R6-7 (www.biomatters.com). Individual gene regions within each data set were first aligned using MAFFT (Katoh & Standly, 2013) and manually inspected.

Phylogenetic analyses

Trees for each gene region were reconstructed using maximum likelihood and Bayesian inference for Involucrata and *Ficus*. Prior to analysis, the best fitting model of sequence evolution was determined using jModeltest v.2.1.4. (Darriba *et al.*, 2012) following the AIC criterion (Posada & Buckley, 2004). In the Involucrata dataset, TIM3+G, TVM+I+G and TIM2+I+G was selected for *G3pdh*, ITS and *GBSSI*, respectively. For *Ficus*, a GTR+G model of sequence evolution was selected for ITS, ETS and *G3pdh*, and TIM2+G, TPM2uf+G and TPM3uf+I+G were selected for *ncpGS*, *GBSSI* and *At103*, respectively. Maximum likelihood analyses were performed in Garli v.2.01.167 (Zwickl, 2006) and repeated five times, each time using a random starting tree and allowing model parameters to be estimated. Support was assessed using 500 bootstrap replicates in Garli (Zwickl, 2006). As these models are nested within the general time reversible model, all matrices were analysed with a GTR+G model for Bayesian analyses. Bayesian analyses were run with MrBayes v.3.2.1 (Huelsenbeck & Ronquist, 2001) for 30 million generations. Stationarity was assessed using

the Trace option in Geneious v.R7 (Biomatters, Ltd) and with Tracer v.1.5 (Rambaut, 2007), and the first 25% of trees sampled in the posterior distribution were removed as burnin.

Before concatenation in a combined analysis, trees were visually inspected and compared for supported (using bootstrap and posterior probabilities) topological congruence. Using PartitionFinder (Lanfear *et al.*, 2012), we determined the best partitioning strategy and models of sequence evolution for the combined datasets. The combined analyses of the *Ficu*s and Involucrata datasets were conducted using the same analysis protocols as described for individual gene regions.

Results

Congruence of phylogenetic trees for involucrata

The ML and Bayesian analyses recovered similar topologies but with different levels of clade support. Bayesian analyses often had higher support for relationships as compared to ML bootstrap analyses (Fig. 1, TreeBase accession S24008). Here, we recovered congruent relationships among the trees with one exception. Subsection *Urostigma* was recovered as monophyletic in the ITS tree [bootstrap (BS) = 97, posterior probability (PP) = 1] but not the *G3pdh* tree (subsection *Urostigma* was not fully sampled in GBSSI tree; Fig. 1, TreeBase accession S24008). As the dedicated analysis of *Ficus* offered an expanded sampling of this clade, a detailed description of relationships recovered in trees resulting from that analysis will be described in the *Ficus* phylogenetic tree section below.

With respect to the Castilleae clade in the Involucrata analyses, *Castilla*, *Helicostylis* Trécul and *Maquira* Aubl. were recovered as monophyletic (Fig.1). *Antiaris* Lesch. and *Poulsenia* are monotypic, and *Antiaropsis* and *Sparattosyce* were each represented by one of the two species. *Naucleopsis* Miq. was recovered as monophyletic in *G3pdh* and ITS trees (Fig. 1, TreeBase accession S24008). However, two clades of *Naucleopsis* spp. were consistently recovered in all trees with one clade containing *N. glabra* Spruce, *N. krukovii* (Standl.) C.C.Berg, *N. ulei* (Warb.) Ducke and *N. imitans* (Ducke) C.C.Berg and a second clade containing *N. caloneura* Ducke, *N. guianensis* (Mildbr.) C.C.Berg *and N. ternstroemiiflora* (Mildbr.) C.C.Berg. *Perebea* Aubl. and *Pseudolmedia* Trécul were not consistently recovered among the trees. The paraphyly of *Perebea* was due to the exclusion of *Perebea mollis* (Poepp. & Endl.) Huber and *P. rubra* (Trécul) C.C.Berg, which formed a clade independent of other *Perebea* spp. (Fig. 1). The core *Perebea* clade often did not include *P. guianensis* Aubl., but there was little support for excluding it. *Pseudolmedia* was recovered as

monophyletic in the *GBSSI* tree and two well-supported *Pseudolmedia* clades were recovered by ITS. These relationships differ as ITS suggested *P. laevis* (Ruiz & Pav.) J.F.Macbr. and *P. macrophylla* Trécul are sister taxa (BS = 100, PP = 1), whereas GBSSI placed *P. laevis* as sister to all *Pseudolmedia* including *P. macrophylla* (BS = 90, PP = 1; Fig. 1, TreeBase accession S24008). *G3pdh* did not recover a clade containing *Psedolmedia* as *P. laevigata* Trécul and *P. rigida* (Klotzsch & H.Karst.) Cuatrec. (which are well-supported sister taxa in all three gene trees) were more closely related to *Perebea mollis* and *P. rubra* (BS = 73, PP = 1; Fig. 1, TreeBase accession S24008).

Few well-supported relationships among genera of Castilleae were recovered in the gene tree analyses. Neotropical taxa were supported as a clade only by ITS (BS = 86, PP = 1; Fig. 1, TreeBase accession S24008), and none of the trees recovered the relationship of the Palaeotropical to Neotropical genera due to lack of resolution. ITS strongly supported a clade containing *Pseudolmedia*, *Perebea*, *Helicostylis* and *Maquira* (BS = 91, PP = 1; Fig. 1, TreeBase accession S24008) and *GBSSI* was unresolved for these nodes. The *G3pdh* tree conflicted with this clade; this tree recovered a clade of *Pseudolmedia*, *Perebea* and *Helicostylis* (BS = 88, PP = 1; Fig. 1, TreeBase accession S24008) to the exclusion of *Maquira*. Instead, *Maquira* was recovered as sister to *Naucleopsis* with moderate to strong support (BS = 71, PP = 0.98). Further, in the clade containing *Psedolmedia*, *Perebea* and *Helicostylis*, the placement of *Pseudolmedia rigida* and *P. laevigata* (as described above) conflicted with both the ITS and *G3pdh* trees.

Combined analysis of involucrata

Although there were supported conflicts when comparing the trees, many of these supported conflicts were only supported by the results of the Bayesian analysis and had low to moderate support in the ML bootstrap analysis. As such, we chose to combine our trees in a total evidence analysis, recognizing that more data will be needed in the future to resolve deeper relationships of the group.

Combining the ITS, *G3pdh* and *GBSSI* data improved the resolution and clade support of the Involucrata phylogenetic tree (Fig. 1). All genera of Castilleae, except *Perebea*, were strongly supported with high bootstrap support and high posterior probabilities (Fig. 1). *Perebea* was recovered as paraphyletic as *P. mollis* and *P. rubra* formed a well-supported clade outside of *Perebea* (BS = 82, PP = 1; Fig. 1) similar to results of the tree analyses. *Antiaropsis decipiens* and *Sparattosyce dioica* were sister taxa (BS = 99, PP = 1; Fig. 1) and formed a clade sister to all other

Castilleae (BS = 86, PP = 0.99; Fig. 1). *Antiaris toxicaria* was recovered as sister to *Mesogyne insignis* BS = 96, PP = 1; Fig. 1), and this clade was recovered as sister to the well-supported clade of Neotropical Castilleae (BS = 94, PP = 1; Fig. 1). In Neotropical Castilleae, *Poulsenia* was recovered as sister to all other Neotropical genera (BS = 91, PP = 1; Fig. 1). Here, *Maquira* was well supported as sister to *Helicostylis*, *Perebea* and *Pseudolmedia* (BS = 78, PP = 1; Fig. 1), similar to the placement in the ITS and *GBSSI* trees. *Pseudolmedia laevigata* and *P. rigida* were recovered in a larger clade of *Pseudolmedia* as opposed to *Perebea rubra* and *P. mollis* as observed in the *G3pdh* tree.

Tree congruence for *Ficus*

The final data set included 307 *Ficus* spp. Numbers of species sampled for each gene region were as follows: *At103* – 140, ETS – 244, ITS – 311, *G3pdh* – 209, *GBSSI* – 60 and *ncpGS* – 79. No strongly supported conflicts between individual datasets were recovered. Individual analysis of the *At103* region provided limited resolution and support but did not conflict with previous findings (phylogenetic reconstruction not shown).

Amplification success of the ETS region was improved considerably using the new *Ficus* specific primer ETS-Fic1 (Supporting Information, Supplementary Table S2) resulting in the addition of 39 new sequences of the ETS region (Supporting Information, Supplementary Table S1). The targeted sampling of ETS using relaxed PCR conditions recovered two copies of the ETS region for several accessions from section *Sycocarpus* (*F. condensa*, *F. fistulosa*, *F. hispida* and *F. scortechinii*) and section *Adenosperma* (*F. adenosperma*). We found that the Hel1 primer used in previous studies preferentially amplified a paralogous copy of ETS for some taxa, which resulted in the polyphyly of subgenus *Sycomorus* recovered in previous studies. Using the new *Ficus* specific primer ETS-Fic1, we successfully amplified the presumably correct copy resulting in new sequences placing section *Sycocarpus* and all members of section *Adenosperma* with the remainder of subgenus *Sycomorus* as supported by ITS and other genes and morphology. Using the ETS-Fic1 primer (Supporting Information, Supplementary Table S2), the new ETS data recovered a monophyletic subgenus *Sycomorus*. All ETS sequences of section *Sycocarpus* and *F. adenosperma* produced prior to this study that represent a paralogous copy were excluded from the data matrix prior to the final analysis.

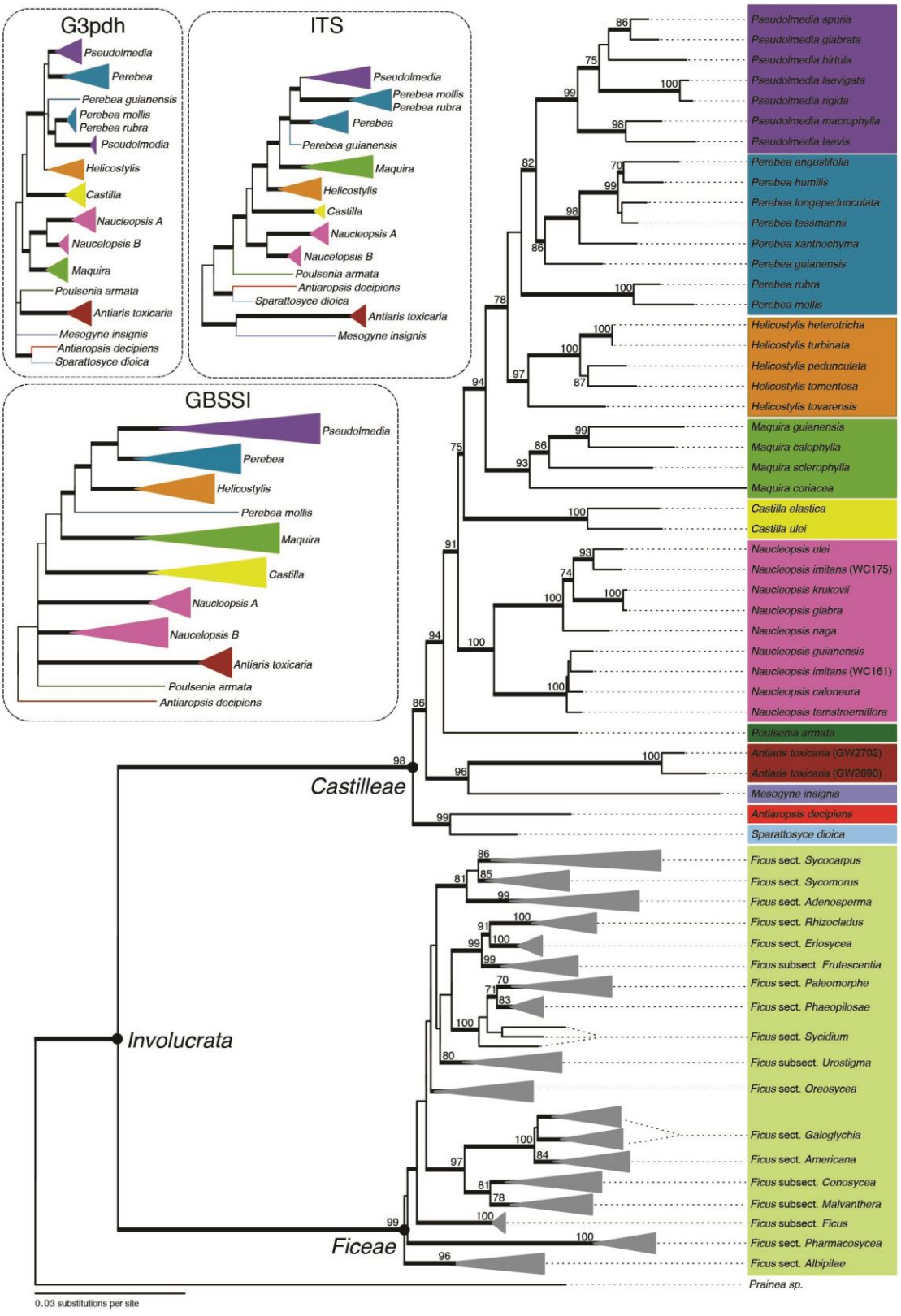

G3pdh
Pseudolmedia
Perebea
Perebea guianensis
Perebea mollis
Perebea rubra
Pseudolmedia
Helicostylis
Castilla
Naucleopsis A
Naucelopsis B
Maquira
Poulsenia armata
Antiaris toxicaria
Mesogyne insignis
Antiaropsis decipiens
Sparattosyce dioica
ITS
Pseudolmedia
Perebea mollis
Perebea rubra
Perebea
Perebea guianensis
Maquira
Helicostylis
Castilla
Naucleopsis A
Naucelopsis B
Poulsenia armata
Antiaropsis decipiens
Sparattosyce dioica
Antiaris toxicaria
Mesogyne insignis
GBSSI
Pseudolmedia
Perebea
Helicostylis
Perebea mollis
Maquira
Castilla
Naucleopsis A
Naucelopsis B
Antiaris toxicaria
Poulsenia armata
Antiaropsis decipiens
Pseudolmedia spuria
Pseudolmedia glabrata
Pseudolmedia hirtula
Pseudolmedia laevigata
Pseudolmedia rigida
Pseudolmedia macrophylla
Pseudolmedia laevis
Perebea angustifolia
Perebea humilis
Perebea longepedunculata
Perebea tessmannii
Perebea xanthochyma
Perebea guianensis
Perebea rubra
Perebea mollis
Helicostylis heterotricha
Helicostylis turbinata
Helicostylis pedunculata
Helicostylis tomentosa
Helicostylis tovarensis
Maquira guianensis
Maquira calophylla
Maquira sclerophylla
Maquira coriacea
Castilla elastica
Castilla ulei
Naucleopsis ulei
Naucleopsis imitans (WC175)
Naucleopsis krukovii
Naucleopsis glabra
Naucleopsis naga
Naucleopsis guianensis
Naucleopsis imitans (WC161)
Naucleopsis caloneura
Naucleopsis ternstroemiflora
Poulsenia armata
Antiaris toxicaria (GW2702)
Antiaris toxicaria (GW2690)
Mesogyne insignis
Antiaropsis decipiens
Sparattosyce dioica
Castilleae
Involucrata
Ficeae
Ficus sect. Sycocarpus
Ficus sect. Sycomorus
Ficus sect. Adenosperma
Ficus sect. Rhizocladus
Ficus sect. Eriosycea
Ficus subsect. Frutescentia
Ficus sect. Paleomorphe
Ficus sect. Phaeopilosae
Ficus sect. Sycidium
Ficus subsect. Urostigma
Ficus sect. Oreosycea
Ficus sect. Galoglychia
Ficus sect. Americana
Ficus subsect. Conosycea
Ficus subsect. Malvanthera
Ficus subsect. Ficus
Ficus sect. Pharmacosycea
Ficus sect. Albipilae
Prainea sp.
0.03 substitutions per site

Figure 1. Phylogenetic trees from individual (upper left panel) and combined (main tree) maximum likelihood analyses of Involucrata using ITS, *G3pdh* and *GBSSI*. Thickened branches represent posterior probabilities greater than 0.95, and maximum likelihood bootstrap values are indicated above the branches (main tree only). Genera in Involucrata are represented by different colours consistent between the trees based on individual loci and the combined phylogenetic tree. In Ficeae, clades corresponding to named sections have been collapsed where possible (full tree not shown). For the three trees, *G3pdh*, ITS and *GBSSI*, clades have been collapsed based on genus or clades with a genus to compare relationships among these groups in each tree (all trees are available in TreeBase accession S24008).

Combined analysis for *Ficus*

The emerging picture of the phylogenetic tree of *Ficus* (Figs 2, 3A–F) was largely consistent with sections or subsections proposed by morphology and provided a coherent global framework, although infrageneric relationships remain uncertain and many relationships were not well supported. The extensive sampling in the present study allowed for interpretation of relationships of several taxa that have been difficult to place using morphology.

Three of the six subgenera (Berg & Corner, 2005), namely *Sycidium* (80% BS/PP = 0.99), *Sycomorus* (97% BS/PP = 1.00) and *Synoecia* (100% BS/PP = 1.00), were monophyletic, whereas subgenera *Ficus*, *Pharmacosycea* and *Urostigma* were polyphyletic. The American section *Pharmacosycea* (100% BS/PP = 1.00) was sister to the remainder of *Ficus* (68% BS/PP = 0.90), although this was not strongly supported. Relationships in the remainder of *Ficus* were not well resolved, but a number of clades were well supported. Section *Oreosycea* (Miq.) Miq. is divided between two clades consisting of subseries *Albipilae* Corner (100% BS/PP = 1.00) and the remainder of section *Oreosycea* (77% BS/ PP = 1.00). Subgenus *Urostigma* is also split into a clade with subsection *Urostigma* (100% BS/ PP = 1.00) and a larger clade (100% BS/PP = 1.00) including the remainder of the former subgenus *Urostigma.* Sections *Urostigma* (Gasp.) Endl. and *Stilpnophyllum* Endl. are polyphyletic. Subgenus *Ficus* is split into three clades corresponding to the *Ficus carica* L. group (100% BS/PP = 1.00), which is unplaced, and sections *Frutescentiae* Sata (92% BS/ PP = 1.00) and *Eriosycea* Miq. (100% BS/PP = 1.00), which form a clade (98% BS/PP = 1.00) together with subgenus *Synoecia* (Miq.) Miq. (100% BS/PP = 1.00).

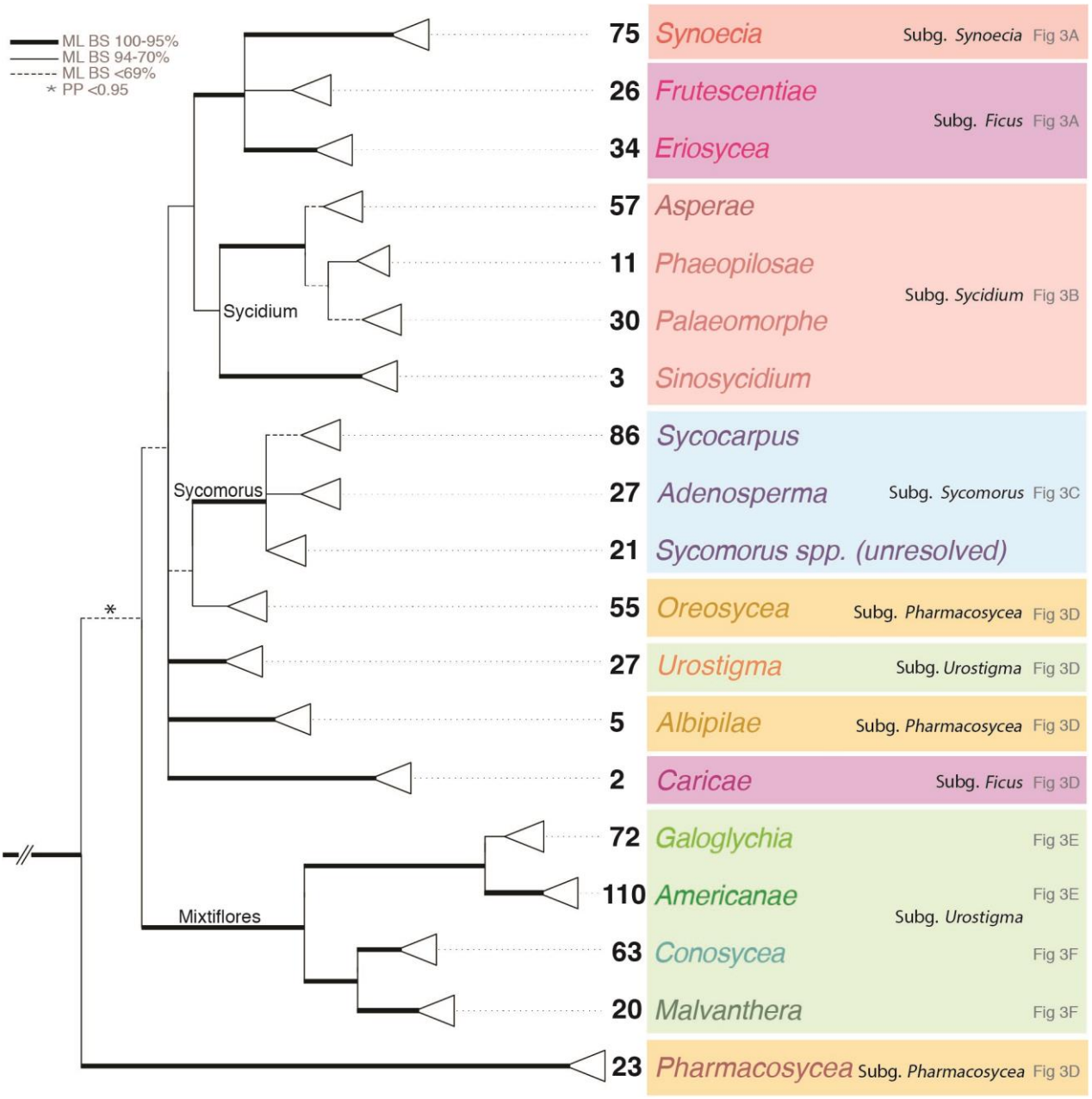


Figure 2. Cladogram based on relationships reconstructed from the maximum likelihood analysis of the six-locus *Ficus* dataset (detailed tree: Fig. 3A–F) providing an overview of the current phylogenetic understanding of relationships in *Ficus*. Approximate number of species in each clade indicated to the left of each clade name, and the subgeneric classification based on Berg & Corner (2005) indicated on the righthand side of the coloured boxes. ML bootstrap support indicated as follows: thickened branch = 95–100%, thin branch = 70–94%, and dashed branches = < 69%; posterior probability > 0.95 indicated with an asterisk.

Discussion

Phylogenetic tree for Involucrata

Here we introduce the name Involucrata to represent the clade containing *Ficus* and Castilleae. With striking variation in numbers of species, genetic diversity and morphology, we discuss differences in historical biogeography, molecular evolution and pollination ecology between *Ficus* and Castilleae to propose future research on evolutionary mechanisms driving the diversification of these two lineages.

The centre of diversity for Castilleae is in the Neotropics, whereas the centre of diversity for *Ficus* is in the Palaeotropics, specifically Borneo and New Guinea (Berg 2005b; Berg *et al.*, 2006). Our study of the phylogenetic tree of Castilleae strongly supports the monophyly of Neotropical Castilleae, suggesting a single colonization event to the New World tropics. In contrast, *Ficus* probably colonized the Neotropics twice, as phylogenetic studies of *Ficus* have recovered two well-supported clades of Neotropical *Ficus* that diversified at different points in evolutionary history (Jousselin *et al.*, 2003; Rønsted *et al.*, 2005; Rønsted *et al.*, 2008a; Cruaud *et al.*, 2012b). Molecular phylogenetic analysis of *Ficus* tentatively identified the Neotropical section *Pharmacosycea* as sister to all other lineages of the genus (Herre *et al.*, 1996; Rønsted *et al.*, 2005; Rønsted *et al.*, 2008a; Cruaud *et al.*, 2012b, BruunLund *et al.*, 2016; Zhang *et al.*, 2018), although the crown group of section *Pharmacosycea* diversified only 16 Mya and long after the origin of *Ficus* at least 75.0–48.5 Mya (Rønsted *et al.*, 2005; Zhang *et al.*, 2018). Estimates of the crown age of Castilleae (50.0– 31.2 Mya) predate the diversification of Neotropical *Ficus* (Rønsted *et al.*, 2005; Zerega *et al.*, 2005; Xu *et al.*, 2011; Cruaud *et al.*, 2012b; Zhang *et al.*, 2018). Differences in the number of colonization events and in the timing of diversification, seen in light of differences in historical climate and biogeographical events (e.g. the Andean uplift; Machado *et al.*, 2018), should inform our comparison of diversification rates between the two lineages.

Highly specific pollination mutualisms, like the fig–fig wasp interaction, have been hypothesized to increase rates of speciation (Stebbins, 1981), although studies in yuccas and yucca moths have shown the opposite (Smith *et al.*, 2008). Pollination syndromes of the sister group (Castillae) are worthy of consideration in terms of how they might influence speciation and extinction (Sakai *et al.*, 2000; Zerega *et al.*, 2004; Moe *et al.*, 2012). It remains unknown if thrips and Castilleae depend on each other for survival, as thrips may be able to breed elsewhere, and Castilleae could receive pollen

from other insects. Research dedicated to assessing the probability of extinction in the two lineages given their pollination syndromes ought to examine the degree to which speciation and extinction rates are associated with diversification (Moe *et al.*, 2012).

If we consider the morphological evolution of figs and Castilleae as it relates to pollination biology, some of the traits associated with the fig–fig wasp pollination mutualism evolved in the common ancestor of *Ficus* and Castilleae (Clement & Weiblen, 2009). For instance, the appearance of an involucre, which is correlated with a shift from wind to insect pollination, occurred prior to the split between *Ficus* and Castilleae (Datwyler & Weiblen, 2004; Clement & Weiblen, 2009). Although the involucre is not exclusive to *Ficus*, tracking subsequent modifications of this trait is important in understanding the evolution of fig pollination where pollinators, hatched in the functional male figs, are part of the male investment of the plant (Anstett, Hossaert-McKey & Kjellberg, 1997). Comparisons of molecular evolutionary rates, morphologies and pollination syndrome are needed to identify factors affecting rates of diversification.

Phylogenetics and Taxonomy of Castilleae

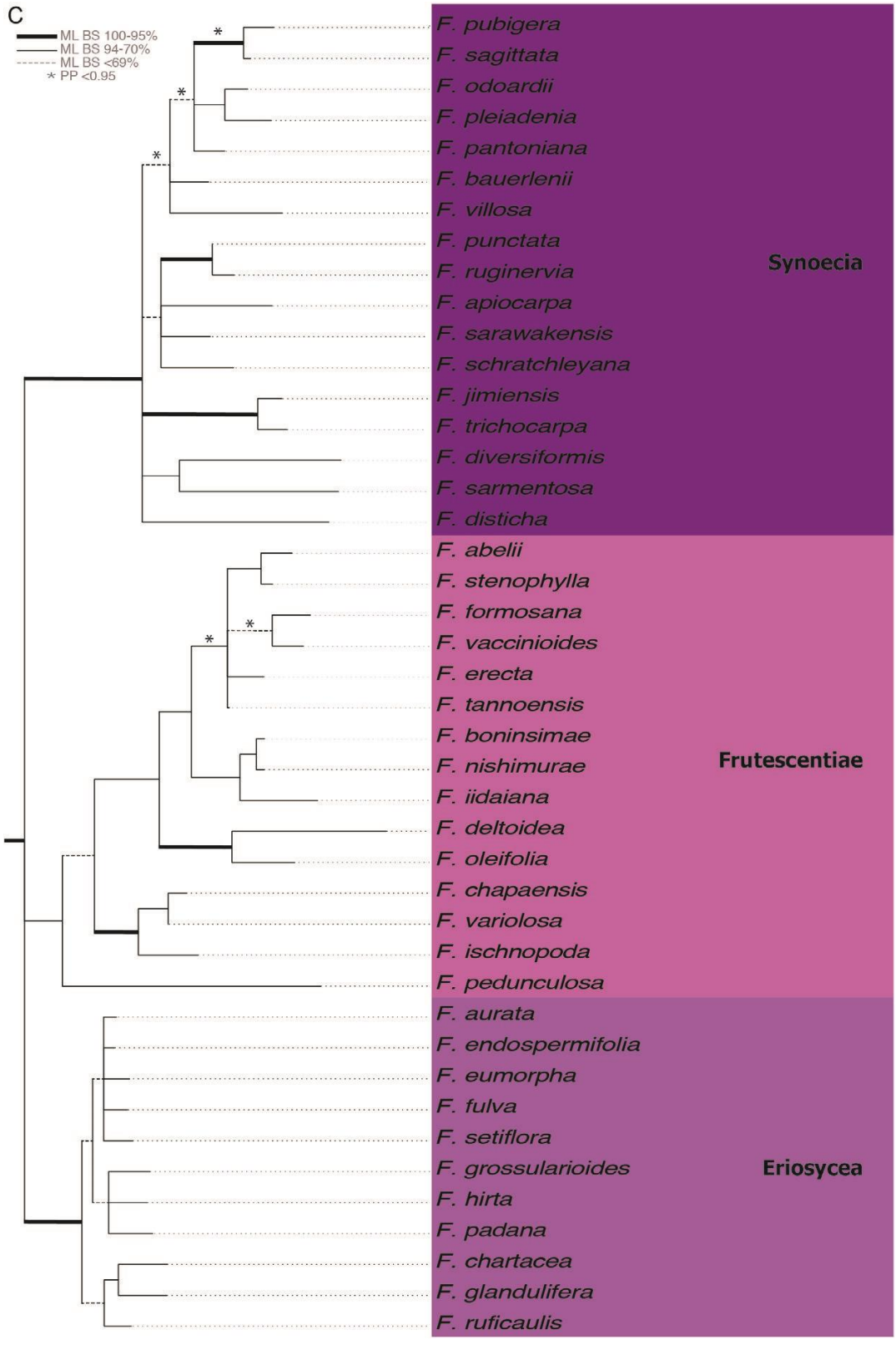
Strong support was recovered for the monophyly of the Neotropical taxa (Fig. 1) also recovered in prior phylogenetic studies of the family (Zerega *et al.*, 2005). In this group, monotypic *Poulsenia* was recovered as sister to all other Neotropical Castilleae. *Poulsenia* has several unique characters that separate it from the remainder of Castilleae including prickles and the loss of septate wood fibres (Berg, 2001).

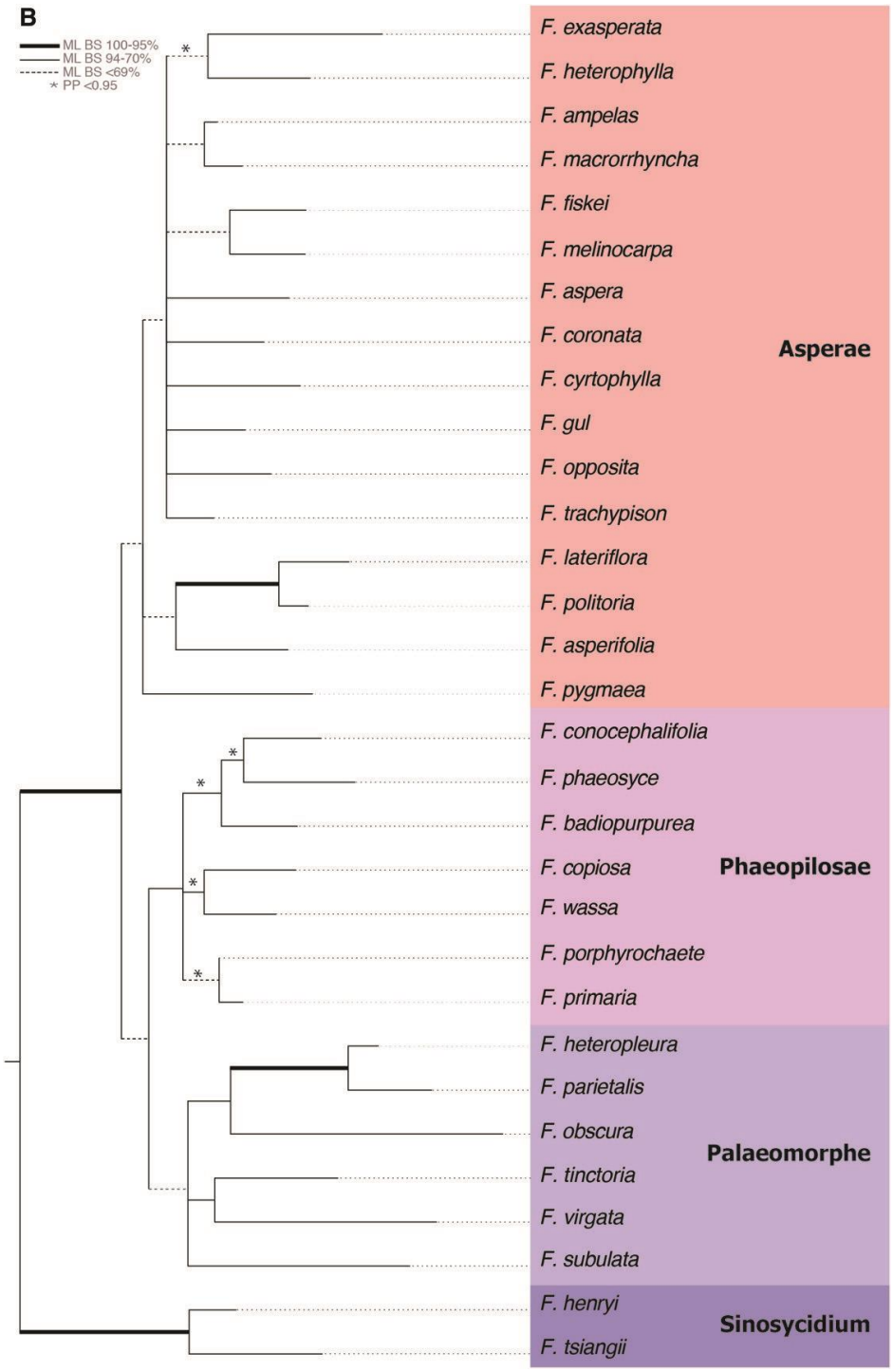
Perebea was consistently recovered as paraphyletic in the individual and combined analyses (Fig. 1, TreeBase accession S24008). *Perebea* section *Noyera* (Trécul) Engl., including *P. rubra* and *P. mollis*, did not group with the rest of the genus. *Noyera* Trécul (Trécul, 1847) was first designated as a genus with the description of *Noyera rubra* Trécul. The genus was later reduced to a section of *Perebea* (Engler, 1889) and also included *P. mollis*. Ducke (1922) reinstated *Noyera* including *N. mollis* (Poepp. & Endl.) Ducke, *N. rubra* and later a third species, *N. glabrifolia* Ducke (Ducke, 1932). In 1972, *Noyera* was again reduced to a section of *Perebea* (Berg, 1972), and *P. rubra* was reduced to a subspecies of *P. mollis.* Later, *P. mollis* subsp. *rubra* (Trécul) C.C.Berg was reinstated as *P. rubra*, and *P. glabrifolia* was reduced to *P. rubra* subsp. *glabrifolia* (Ducke) C.C.Berg (Berg, 2001). Section *Noyera* differs from the rest of *Perebea* in having pluricellular globose capitate hairs on the lower leaf surface, filiform stigmas and inner involucral bracts that are long and incurved

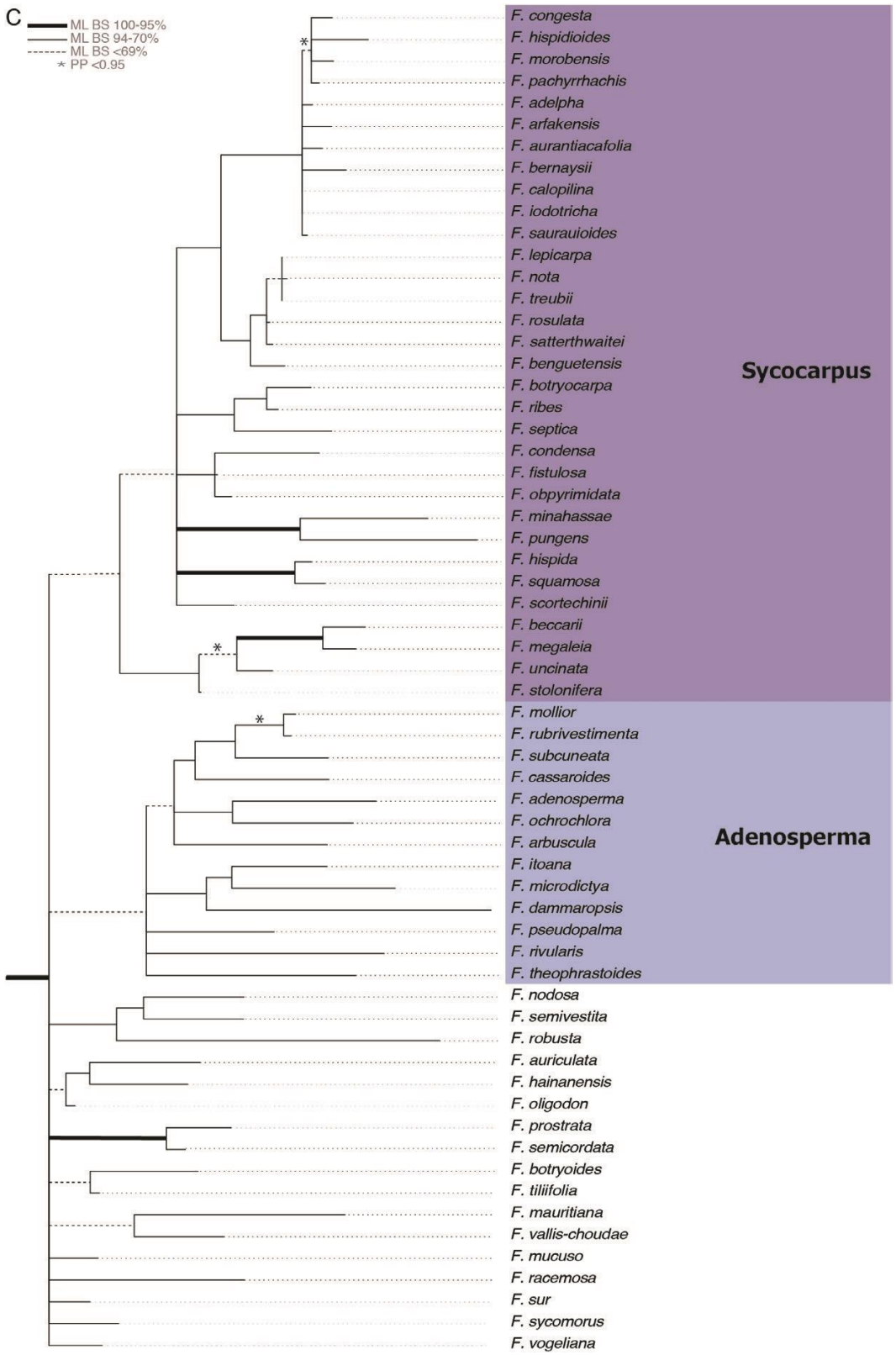
prior to anthesis (Berg, 1972, 2001). Based on molecular evidence and these diagnostic features, we recommend reinstating the genus *Noyera* with *N. mollis* and *N. rubra* as the sole members. An alternative taxonomic proposal would be to expand the circumscription of *Perebea* to encompass *Pseudolmedia*. However, *Pseudolmedia*, has recognizably distinct morphology that supports maintaining it as a genus for practical reasons. All *Pseudolmedia* spp. are dioecious with uniflorous pistillate inflorescences (Berg, 1972, 1977, 2001). Further, ITS and *GBSSI* phylogenetic trees support the monophyly of *Pseudolmedia*, but the *G3pdh* tree recovered a paraphyletic *Pseudolmedia*. Although more data are needed to investigate this conflict among trees, the relationships recovered by the ITS and GBSSI trees, not *G3pdh*, are corroborated by morphology.

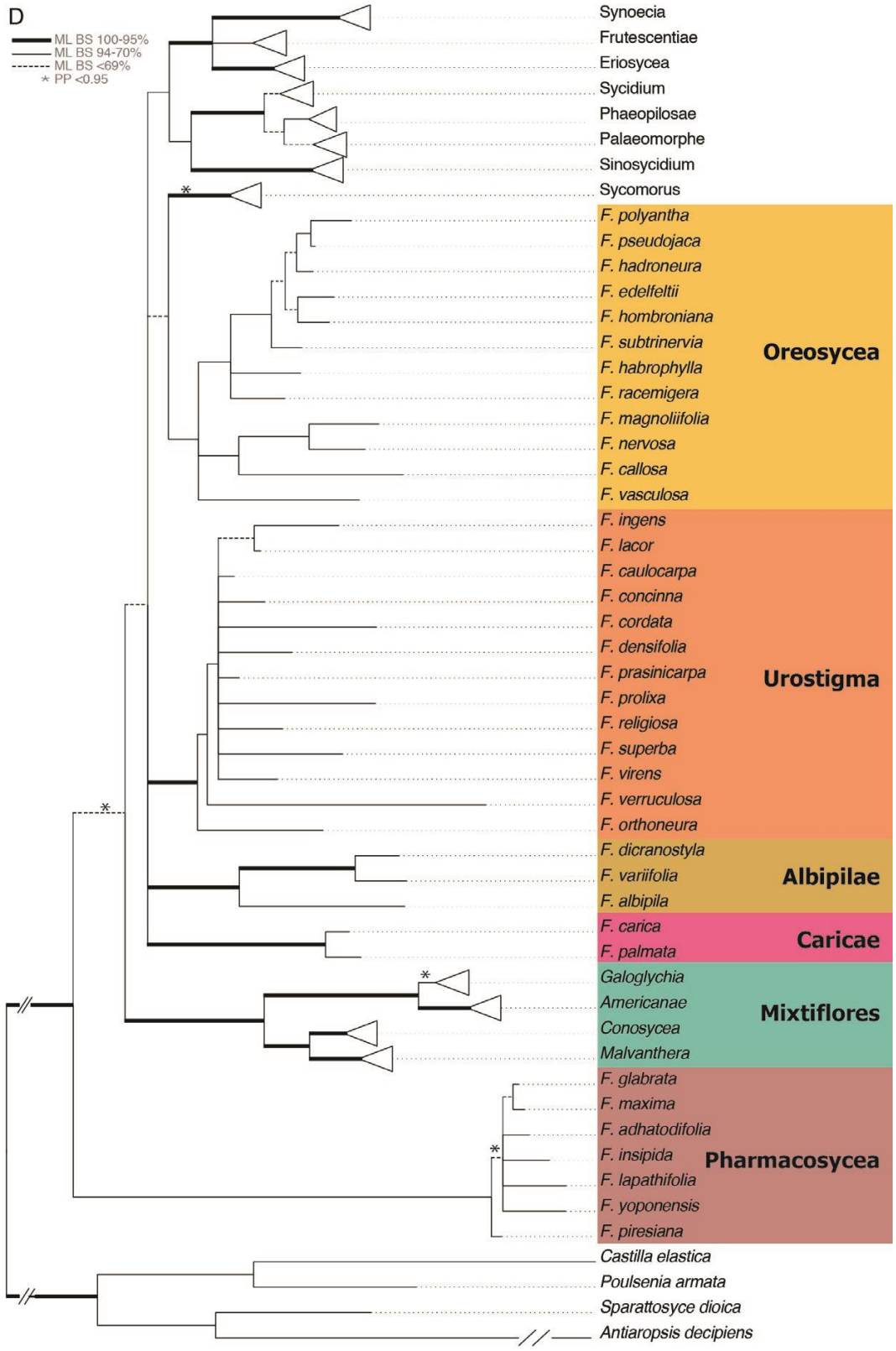
Our analysis supported the monophyly of *Helicostylis* and confirmed the position of the morphologically distinct *H. tovarensis* (Klotzsch & H.Karst) C.C.Berg as sister to all other *Helicostylis* (Fig. 1). *Helicostylis tovarensis* differs from the rest of the genus on account of free rather than basally connate tepals in pistillate flowers, which are uniflorous rather than multiflorous, and one or two staminate inflorescences per leaf axil (Berg, 1972).

Figure 3A–F. Maximum likelihood tree of the combined analysis of six gene regions for 307 *Ficus* spp. ML bootstrap support indicated as follows: thickened branches = 95–100%, thin branches = 70–94% and dashed branches = < 69%; posterior probability > 0.95 indicated with an asterisk. Species included in phylogenetic analysis of *Ficus* for the first time marked in bold. Proposed names for monophyletic groups of figs are indicated to the right of each clade throughout the figure. *A. Synoecia*, Frutescentiae and Eriosycea. *B. Asperae*, Phaeopilosae, Palaeomorphe and Sinosycidium. *C. Sycocarpus*, Adenosperma and Sycomorus spp. *D. Oreosycea*, Urostigma, Albipilae, Caricae, and Pharmacosycea. *E. Galoglychia* and Americanae. *F. Conosycea* and Malvanthera.

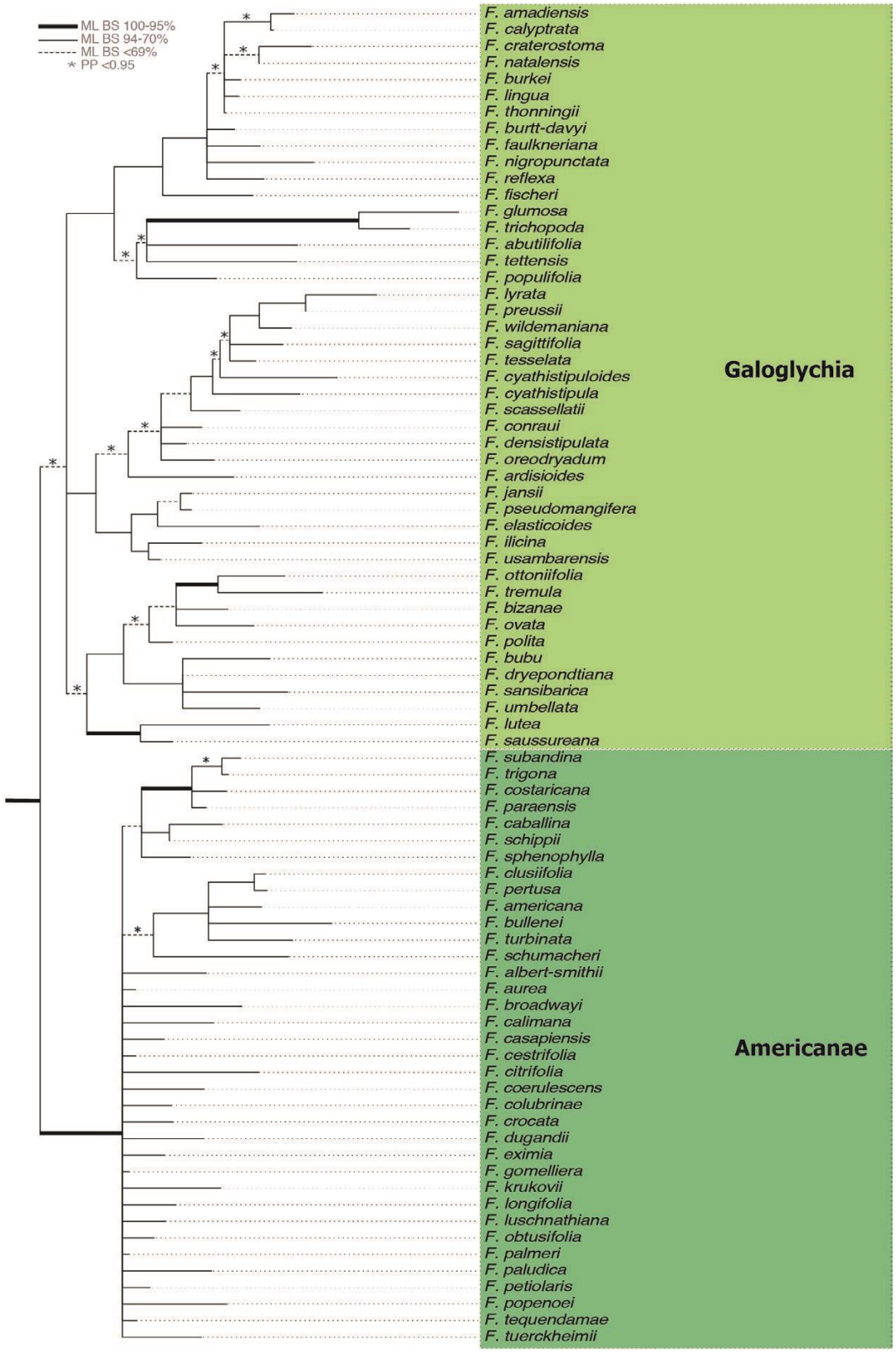

C
ML BS 100-95%
ML BS 94-70%
ML BS <69%
* PP <0.95
F. pubigera
F. sagittata
F. odoardii
F. pleiadenia
F. pantoniana
F. bauerlenii
F. villosa
F. punctata
F. ruginervia
F. apiocarpa
F. sarawakensis
F. schratchleyana
F. jimiensis
F. trichocarpa
F. diversiformis
F. sarmentosa
F. disticha
Synoecia
F. abelii
F. stenophylla
F. formosana
F. vaccinioides
F. erecta
F. tannoensis
F. boninsimae
F. nishimurae
F. iidaiana
F. deltoidea
F. oleifolia
F. chapaensis
F. variolosa
F. ischnopoda
F. pedunculosa
Frutescentiae
F. aurata
F. endospermifolia
F. eumorpha
F. fulva
F. setiflora
F. grossularioides
F. hirta
F. padana
F. chartacea
F. glandulifera
F. ruficaulis
Eriosycea

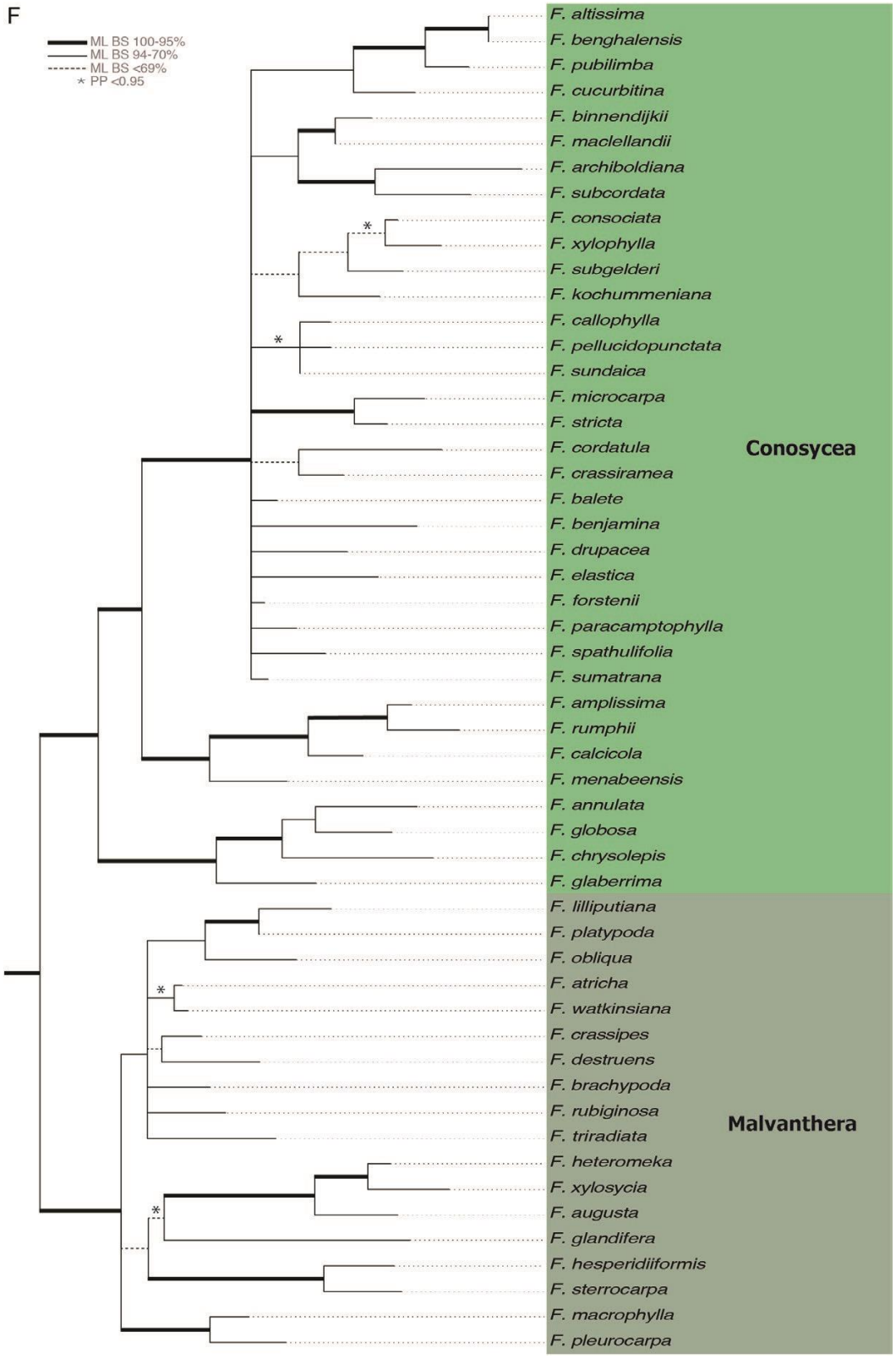

B
ML BS 100-95%
ML BS 94-70%
ML BS <69%
* PP <0.95
F. exasperata
F. heterophylla
F. ampelas
F. macrorrhyncha
F. fiskei
F. melinocarpa
F. aspera
F. coronata
F. cyrtophylla
F. gul
F. opposita
F. trachypison
F. lateriflora
F. politoria
F. asperifolia
F. pygmaea
Asperae
F. conocephalifolia
F. phaeosyce
F. badiopurpurea
F. copiosa
F. wassa
F. porphyrochaete
F. primaria
Phaeopilosae
F. heteropleura
F. parietalis
F. obscura
F. tinctoria
F. virgata
F. subulata
Palaeomorphe
F. henryi
F. tsiangii
Sinosycidium


C
ML BS 100-95%
ML BS 94-70%
ML BS <69%
* PP <0.95
F. congesta
F. hispidioides
F. morobensis
F. pachyrrhachis
F. adelpha
F. arfakensis
F. aurantiacafolia
F. bernaysii
F. calopilina
F. iodotricha
F. saurauioides
F. lepicarpa
F. nota
F. treubii
F. rosulata
F. satterthwaitei
F. benguetensis
F. botryocarpa
F. ribes
F. septica
F. condensa
F. fistulosa
F. obpyrimidata
F. minahassae
F. pungens
F. hispida
F. squamosa
F. scortechinii
F. beccarii
F. megaleia
F. uncinata
F. stolonifera
Sycocarpus
F. mollior
F. rubrivestimenta
F. subcuneata
F. cassaroides
F. adenosperma
F. ochrochlora
F. arbuscula
F. itoana
F. microdictya
F. dammaropsis
F. pseudopalma
F. rivularis
F. theophrastoides
Adenosperma
F. nodosa
F. semivestita
F. robusta
F. auriculata
F. hainanensis
F. oligodon
F. prostrata
F. semicordata
F. botryoides
F. tiliifolia
F. mauritiana
F. vallis-choudae
F. mucuso
F. racemosa
F. sur
F. sycomorus
F. vogeliana


D
ML BS 100-95%
ML BS 94-70%
ML BS <69%
* PP <0.95
Synoecia
Frutescentiae
Eriosycea
Sycidium
Phaeopilosae
Palaeomorphe
Sinosycidium
Sycomorus
F. polyantha
F. pseudojaca
F. hadroneura
F. edelfeltii
F. hombroniana
F. subtrinervia
F. habrophylla
F. racemigera
F. magnoliifolia
F. nervosa
F. callosa
F. vasculosa
Oreosycea
F. ingens
F. lacor
F. caulocarpa
F. concinna
F. cordata
F. densifolia
F. prasinicarpa
F. prolixa
F. religiosa
F. superba
F. virens
F. verruculosa
F. orthoneura
Urostigma
F. dicranostyla
F. variifolia
F. albipila
Albipilae
F. carica
F. palmata
Caricae
Galoglychia
Americanae
Conosycea
Malvanthera
Mixtiflores
F. glabrata
F. maxima
F. adhatodifolia
F. insipida
F. lapathifolia
F. yoponensis
F. piresiana
Pharmacosycea
Castilla elastica
Poulsenia armata
Sparattosyce dioica
Antiaropsis decipiens

E


ML BS 100-95%
ML BS 94-70%
ML BS <69%
* PP <0.95
F. amadiensis
F. calyptrata
F. craterostoma
F. natalensis
F. burkei
F. lingua
F. thonningii
F. burtt-davyi
F. faulkneriana
F. nigropunctata
F. reflexa
F. fischeri
F. glumosa
F. trichopoda
F. abutilifolia
F. tettensis
F. populifolia
F. lyrata
F. preussii
F. wildemaniana
F. sagittifolia
F. tesselata
F. cyathistipuloides
F. cyathistipula
F. scassellatii
F. conraui
F. densistipulata
F. oreodryadum
F. ardisioides
F. jansii
F. pseudomangifera
F. elasticoides
F. ilicina
F. usambarensis
F. ottoniifolia
F. tremula
F. bizanae
F. ovata
F. polita
F. bubu
F. dryepondtiana
F. sansibarica
F. umbellata
F. lutea
F. saussureana
Galoglychia
F. subandina
F. trigona
F. costaricana
F. paraensis
F. caballina
F. schippii
F. sphenophylla
F. clusiifolia
F. pertusa
F. americana
F. bullenei
F. turbinata
F. schumacheri
F. albert-smithii
F. aurea
F. broadwayi
F. calimana
F. casapiensis
F. cestrifolia
F. citrifolia
F. coerulescens
F. colubrinae
F. crocata
F. dugandii
F. eximia
F. gomelliera
F. krukovii
F. longifolia
F. luschnathiana
F. obtusifolia
F. palmeri
F. paludica
F. petiolaris
F. popenoei
F. tequendamae
F. tuerckheimii
Americanae


F
ML BS 100-95%
ML BS 94-70%
ML BS <69%
* PP <0.95
F. altissima
F. benghalensis
F. pubilimba
F. cucurbitina
F. binnendijkii
F. maclellandii
F. archiboldiana
F. subcordata
F. consociata
F. xylophylla
F. subgelderi
F. kochummeniana
F. callophylla
F. pellucidopunctata
F. sundaica
F. microcarpa
F. stricta
F. cordatula
F. crassiramea
F. balete
F. benjamina
F. drupacea
F. elastica
F. forstenii
F. paracamptophylla
F. spathulifolia
F. sumatrana
F. amplissima
F. rumphii
F. calcicola
F. menabeensis
F. annulata
F. globosa
F. chrysolepis
F. glaberrima
F. lilliputiana
F. platypoda
F. obliqua
F. atricha
F. watkinsiana
F. crassipes
F. destruens
F. brachypoda
F. rubiginosa
F. triradiata
F. heteromeka
F. xylosycia
F. augusta
F. glandifera
F. hesperidiiformis
F. sterrocarpa
F. macrophylla
F. pleurocarpa
Conosycea
Malvanthera

Although a combined analysis strongly supported the monophyly of all genera of Castilleae except *Perebea* (and apart from the three monotypic genera, *Poulsenia*, *Antiaris* and *Mesogyne* Engl.), tree analysis of the Involucrata data set shed light on a number of conflicts. As the analysis was based on just two low-copy nuclear genes and the internal transcribed spacer region of ribosomal DNA, there is much room for conflict among diverging trees. Specifically, the placement of *Maquira* and the monophyly of *Pseudolmedia* were called to question by *G3pdh* (Fig. 1). We speculate that the *G3pdh* tree is discordant with a Castilleae species tree based on nuclear ITS, *GBSSI*, 26S (Zerega *et al.*, 2005; Zerega, Nur Supardi & Motley, 2010), plastid *ndhF* region (Datywler & Weiblen, 2004) and morphology. Although the source of the conflict is unknown at this time, some possibilities include having sampled a divergent allele or paralogue for *Maquira*. Regardless, use of this gene region in the future will require further investigation of the *G3pdh* gene history in Involucrata. Other conflicts were observed but supported only by Bayesian posterior probabilities that have been shown to consistently over estimate branch support (Huelsenbeck *et al.*, 2002; Erixon *et al.*, 2003).

Phylogenetics and taxonomy of *Ficus*

Compared to the most recent comprehensive phylogenetic studies (Xu *et al.*, 2011; Cruaud *et al.*, 2012b), the present study increased taxon sampling by 42 species that were not included in any of the previous studies, introduced data from a gene region, AT103 (new to phylogenetic studies of *Ficus*), and reduced the amount of missing data in the matrix adding *c*. 140 new sequences for *Ficus*. The topology obtained from the At103 region was consistent with prior phylogenetic studies of *Ficus* (e.g. Cruaud et al., 2012b). Of the *Ficus* spp. included for the first time here (highlighted in bold, Fig. 3A–F), most are placed in the same clades as their closest relatives predicted from their current classification *sensu* Berg & Corner (2005). The inclusion and verification of the placement of these taxa in a comprehensive phylogenetic framework provides stronger evidence for the current circumscription of clades and infrageneric relationships of *Ficus.*

Some taxa that have been difficult to classify based on their morphology were also included in this phylogenetic analysis of *Ficus* for the first time. For example, inclusion of additional taxa from subgenus *Sycidium* including *F. tsiangii* Corner as a second representative of the *Sinosycidium* group (section *Sinosycidium* Corner) helped to confidently identify four major subclades of subgenus *Sycidium* (groups *Palaeomorphe*, *Phaeopilosae*, *Sinosycidium* and *Sycidium*; Fig. 3D). On the other hand, additional sampling of the *Oreosycea* and *Synoecia* clades highlighted the need for further

revision of these groups as emerging subclades do not reflect the current morphological classification (Fig. 3A, B). Taxonomic implications of this most comprehensive phylogenetic framework are discussed next.

Current clades to guide the classification of F*icus*

The comparison of morphology-based classification to phylogenetic reconstruction of evolutionary relationships among *Ficus* identified taxonomic revisions that are needed to guide future evolutionary studies of the clade. Whether the use of rank-based or rank-free taxonomy is applied to future revisions of *Ficus*, applying names to monophyletic groups should be central to either approach. In our species sampling of *Ficus*, we attempted to include the type species of former sections to help circumscribe clades. However, this was not always possible; in such cases, we relied on identifying clades based on classically accepted concepts of sections. Ultimately, we propose the recognition of a number of clades in *Ficus* that in some cases reinforce the classification of Berg & Corner (2005) and in other cases depart from it to provide clarity and precision when communicating about *Ficus* diversity.

The set of clade names proposed here more accurately recognizes the evolutionary history of *Ficus*. Wherever possible, we applied names historically associated with groups of *Ficus*, and in some cases (e.g. Mixtiflores) new names were proposed for new assemblages of species. Each clade name is presented in conjunction with the closest Linnaean name and rank when possible for comparison to prior publications on *Ficus* classification. Figure 2 should be referenced for interpreting the relationships and hierarchy of the clades presented in the following discussion. Although we do not formally revise fig taxonomy here as further resolution and support for many clades are wanting, we encourage future revisionary work to consider a rankfree taxonomy given the number of clades researchers would want to regularly discuss due to the size and complex evolutionary history of the group (e.g. shifts in breeding system, pollinator behaviour, habit etc.).

Synoecia

This clade (Fig. 3A; 100% BS/PP = 1.00) corresponds to *Ficus* subgenus *Synoecia* (Miq.) Miq., one of the three subgenera that are monophyletic. This clade includes *c*. 72 species of dioecious root climbers in Asia and Australasia (Berg, 2003d; Berg & Corner 2005). Berg & Corner (2005) subdivided *Synoecia* into sections *Rhizocladus* Endl. (primarily in New Guinea) and *Kissosycea*

Miq. (primarily in Borneo), which are not clear-cut based on morphology; these sections are not resolved by the present molecular study. Notably, there is a clade consisting of *F. sarmentosa* Buch.-Ham. ex Sm. and *F. diversiformis* Miq. *Ficus sarmentosa* is traditionally considered a member of section *Rhizocladus*, but is a variable species with affinities to the *Punctata* group of section *Kissosycea* (Berg & Corner, 2005). *Ficus diversiformis* is traditionally considered a member of the Malesian section *Kissosycea*, but it is one of only two species confined to mainland Asian (Berg & Corner, 2005). The other species, *F. hederacea* Roxb., was not sequenced for this study. *Ficus pumila* L. is also a root climber traditionally included in section *Rhizocladus*, but previous studies (e.g. Rønsted, 2008a) have shown that *F. pumila* is more closely related to traditional *Ficus* spp. of section *Frutescentiae* (subgenus *Ficus*), showing that the root-climbing habit has evolved at least twice. A few other root climbers such as the essentially Sino–Himalayan *F. laevis* Desf. and *F. pubigera* (Wall. ex Miq.) Miq. also show affinities to members of subgenus *Ficus* (Berg & Corner, 2005). *Ficus laevis* was not sequenced for this study, but *F. pubigera* is imbedded in section *Rhizocladus*.

Frutescentiae

This clade (Fig. 3A; 92% BS/PP = 0.87) corresponds to section *Ficus* subsection *Frutescentiae* Sata and consists of 25–30 species including *F. pumila* and *F. iidaiana* Wilson, mostly from the Sino–Himalayan region and eight species from western Malesia. The *Frutescentiae* clade is closely related to the *Eriosycea* and *Synoecia* clades.

Eriosycea

This clade (Fig. 3A; 100% BS/PP = 1.00) corresponds to section *Eriosycea* Miq. with *c*. 34 species ranging from Sino–Himalaya to New Guinea. The *Eriosycea* and *Frutescentiae* clades are closely related to the *Synoecia* clade and together this group forms a well-supported clade (Fig. 3A; 98% BS/PP = 0.98), which has also been resolved in previous studies. However, subgenus *Ficus* to which Frutescentiae and Eriosycea have been placed, is polyphyletic on account of the position of section *Ficus* (see the discussion on the Caricae clade).

Sycidium

This clade (Figs. 2, 3B; 80% BS/PP = 0.81) corresponds to subgenus *Sycidium* (Miq.) Berg & Corner, which is another of the three monophyletic subgenera of *Ficus.* Sycidium includes *c*. 110

dioecious species primarily in Asia and Australasia with approximately ten species in Africa and Madagascar (Berg, 2003e; Berg & Corner, 2005). The *Sycidium* clade also largely corresponds to section *Sycidium sensu* Corner 1965, but excluding series *Pungentes* Corner [*F. minnahassae* (Teifjsm. & de Vriese) Miq. and *F. pungens* Reinw. ex Blume], which Berg transferred to subgenus *Sycomorus*, and including section *Sinosycidium* and series *Sinosyceae* (Berg, 2003e). Berg (2003e) subdivided subgenus *Sycidium* into two sections based primarily on differences in growth habit and the flowers; section *Palaeomorphe* King with aerial adventitious roots and hermaphroditic flowers with ovules galled by pollinators, and section *Sycidium* without aerial adventitious roots. In the present study, four major clades are recognized, which may be ranked as sections if stronger support is obtained in the future (*Palaeomorphe Phaeopilosae*, *Sinosycidium* and *Asperae* clades). Three Asian mainland species constituting section *Sinosycidium* are sister to the remaining subclades.

Asperae

This clade (Fig. 3B; 55% BS/PP = 0.56) corresponds to section *Sycidium* (Miq.) Berg & Corner, excluding *Phaeopilosae* (King) Corner and *Sinosycidium* Corner. We recommend referring to this clade as Asperae rather than Sycidium to reduce confusion because this clade is nested in the larger clade Sycidium (Fig. 2). The name Asperae refers to *F. aspera*, the type species of subgenus *Sycidium* being including in the former section *Sycidium*. The delimitation of this clade and its subdivisions may need revision once data including more species becomes available.

Phaeopilosae

This constitutes a well-supported clade (Fig. 3B; 92% BS/PP = 0.91) of species endemic to New Guinea and tropical Australia largely corresponding to the *Conocephalifolia* group *sensu* Berg including *F. wassa* Roxb. and *F. copiosa* Steud. but excluding *Ficus gul* Lauterb. & K.Schum. As a result, the *Phaeopilosae* clade is confined to Eastern New Guinea and North Queensland. *Ficus complexa* Corner, the type species for Corner's series *Phaeopilosae*, as well as a number of other species included in Corner's series *Phaeopilosae* or in Bergs *Conocephalifolia* group were not included in this study so that the circumscription and name of the *Phaeopilosae* clade is uncertain at present.

Palaeomorphe

This clade (Fig. 3B; 60% BS/PP = 0.65) corresponds to section *Palaeomorphe* (King) Berg & Corner and includes *c*. 30 species of climbers or hemi-epiphytes with aerial adventitious roots. The name refers to the frequent presence of hermaphroditic flowers instead of male ones, with an ovule capable of becoming a gall.

Sinosycidium

This clade (Fig. 3B; 100% BS/PP = 1.00) corresponds to the monotypic Chinese section *Sinosycidium* Corner (*F. tsiangii*) and subsection *Ficus* series *Sinosycea* Corner comprising *F. henryi* Diels and *F. subincisa* Sm. from mainland Asia. *Ficus subincisa* was not included in this study. The species of section *Sinosycidium* are atypical in *Sycidium* in that they present elongate stigmas in female figs and two anthers per male flower in male figs, two traits probably linked to being passively pollinated. Passive pollination has not been reported for any other species of subgenus *Sycidium*.

Sycomorus

This clade (Fig. 3C; 97% BS/PP = 1.00) corresponds to subgenus *Sycomorus* (Gasp.) Miq., which is the final subgenus of *Ficus* supported as monophyletic in phylogenetic reconstructions. Sycomorus includes members of sections *Sycomorus s.l.* (18 species including former section *Neomorphe*), *Sycocarpus* (86 species) and *Adenosperma* (20 species). In addition, this group includes a number of smaller sections (*sensu* Berg & Corner, 2005) with difficult affinities, namely *Dammaropsis* (Warb.) C.C.Berg (five species), *Hemicardia* C.C.Berg (three species), *Papuasyce* (Corner) C.C.Berg (three species) and *Bosscheria* (Teijsm. & de Vriese) C.C.Berg (two species). Corner (1965) only included the monoecious section *Sycomorus* in subgenus *Sycomorus*. However, based on early molecular studies (Weiblen 2000; Jousselin *et al.*, 2003), morphological evidence (Corner, 1967; Berg, 1989; Weiblen, 2000) and a shared genus of pollinating wasps (*Ceratosolen*), Berg & Corner (2005) transferred a number of dioecious sections from Corner's (1965) subgenus *Ficus* into an enlarged subgenus *Sycomorus*, which we here refer to as the Sycomorus clade.

Two preceding molecular studies including more taxa (Rønsted et al., 2005, 2008a) did not find support for such an expanded subgenus *Sycomorus*, but this was attributed to lack of resolution and informative characters using limited DNA sequence information. Undiscovered paralogous copies of ETS were problematic in Rønsted *et al.* (2005, 2008a). Here we have identified and removed

erroneous copies of ETS and included homologous ETS sequences for this group; as a result, *Sycomorus* was recovered as monophyletic.

Relationships in the *Sycomorus* clade were not well supported in this study and are likely to change with future analyses, but we would expect to recover clades largely corresponding to sections *Sycomorus s.l.*, *Sycocarpus* and *Adenosperma* once the many difficult taxa in the subgenus *Sycomorus* clade are placed. Sections *Sycocarpus* and *Adenosperma* are both resolved with low support. Section *Sycomorus s.l.* is not resolved (Fig. 3C), and we therefore refrain from informally naming these clades at this time.

Section *Papuasyce* of Berg & Corner (2005) includes three species, *F. itoana* Diels and *F. microdictya* Diels endemic to New Guinea and New Britain and *F. pritchardii* Seem. endemic to Fiji (Berg & Corner, 2005). Section *Papuasyce* was listed as subsection *Papuasyce* in section *Sycocarpus* by Corner (1965). Section *Papuasyce* and section *Adenosperma* lack the nodal glands typical of section *Sycocarpus* Berg & Corner (2005). The dioecious *F. itoana* and the monoecious *F. microdictya* are sisters in the present study, whereas *F. pritchardii* was not included.

Section *Dammaropsis* includes five species, *F. dammaropsis* Diels, *F. pseudopalma* Blanco, *F. rivularis* Merr., *F. solomonensis* Rech. and *F. theophrastoides* Seem. ranging from the Philippines to the Solomon Islands. Corner (1965) placed *F. dammaropsis* as subsection *Dammaropsis* and *F. solomonensis* and *F. theophrastoides* in subsection *Auriculisperma*, as series *Theophrastoides* in section *Sycocarpus*. *Ficus pseudopalma* and *F. rivularis* was included as series *Pseudopalmae* and *Rivulares* respectively in subsection *Ficus* by Corner (1965). In the present analysis, all of these species except *F. solomonensis* are included and their relationship is unresolved among members of section *Adenosperma* of Berg & Corner (2005b), with which they share spirally and terminally arranged and more or less conspicuously tufted leaves (Berg, 2004a; Berg & Corner, 2005).

Section *Hemicardia* of Berg & Corner (2005) was originally described as series *Prostratae* in section *Sycidium* (subgenus *Sycidium*; Corner, 1965). Section *Hemicardia* is supported by free tepals, and one or two anthers per male flower, is primarily Sino–Himalayan and includes *F. koutumensis* Corner, *F. prostrata* (Wall. ex. Miq.) Miq. and *F. semicordata* Buch.-Ham. ex Sm., the latter extending to Malesia. Berg (2004a) noted the closer morphological affinities of section *Hemicardia* to section *Sycomorus* than to any of the other sections of the subgenus. In the present analysis, *F.*

koutumensis is not included, but *F. prostrata* and *F. semicordata* form a clade (Fig. 3C; 98% BS/PP = 1.00) with uncertain affinity.

Section *Bosscheria* of Berg & Corner (2005) includes *F. minnahassae* and *F. pungens* ranging from the Philippines to New Guinea. Section *Bosscheria* of Berg & Corner (2005) forms a clade, which is embedded in the *Sycocarpus* group in the present analysis. They are atypical in the subgenus because of their small figs and flowers.

Sycocarpus

This clade (Fig. 3C; 68% BS/PP = 0.71) corresponds to section *Sycocarpus* Miq and includes 86 species.

Adenosperma

This clade (Fig. 3C; 68% BS/PP = 0.51) largely corresponds to section *Adenosperma* Corner and comprises 20 species.

Oreosycea

This clade (Fig. 3D; 77% BS/PP = 0.62) corresponds to the Palaeotropical section *Oreosycea* (Miq.). Miq. tentatively including most of subsections *Glandulosae* C.C.Berg and *Pedunculatae* Sata *sensu* Berg & Corner (2005), but excluding subseries *Albipilae* (Berg, 2003a; Berg & Corner, 2005). Corner (1959) placed section *Oreosycea* in subgenus *Pharmacosycea* (Miq.) Miq, but molecular phylogenetic evidence has suggested section *Oreosycea* is more closely related to subgenus *Sycomorus*; however, this is not well-supported (54% BS/PP < 0.50 in this study) or consistent. Berg & Corner (Berg, 2003b; Berg & Corner, 2005) divided section *Oreosycea* into subsections *Glandulosa*e C.C.Berg (including series *Austrocaledonicae* Corner) and series *Nervosae* Corner and *Pedunculatae* (including subseries *Vasculosae* Corner and subseries *Albipilae* Corner).

Urostigma

This clade (Fig. 3D; 100% BS/PP = 0.99) corresponds to section *Urostigma sensu* Corner 1960. Due to the placement of section *Urostigma* in this phylogenetic analysis and prior studies of *Ficus* (Jousselin *et al.*, 2003; Rønsted et al., 2005, 2008a), subgenus *Urostigma* is polyphyletic. The Urostigma clade should be recognized independently from the remaining sections of the former subgenus *Urostigma* (refer to the Mixtiflores discussion). Additionally, Berg & Corner (2005)

expanded section *Urostigma* uniting Corner's sections *Urostigma, Leucogyne* and *Conosycea*, which is not supported by this study. The Sino–Himalayan *F. orthoneura* H.Lév. & Vanoit appears to be sister to the rest of (sub)section *Urostigma* (100% BS/PP = 1.00). *Ficus orthoneura*, *F. hookeriana* Corner (also Sino–Himalayan, but not included in this study) and *F. cornelisiana* Chantaras & Y.Q.Peng (Chanterasuwan et al., 2014) present a mixture of characters of section *Urostigma* and section *Conosycea* and were placed in their own series in section *Urostigma* by Corner (1965). In a recent study of (sub)section *Urostigma* (Chantarasuwan *et al.*, 2015), *F. madagascariensis* C.C.Berg (not included here) was found to be sister to the remainder of the (sub)section and the next diverging clade consisted of *F. orthoneura* and *F. hookeriana*.

Albipilae

This clade (Fig. 3A; 100% BS/PP = 1.00) corresponds to subseries *Albipilae* Corner and comprised two African species, *F. variifolia* Warb. and *F. dicranostyla* Mildbr., and *F. albipila* (Miq.) King that occurs from Thailand to Australia. Morphological study of subseries *Albipilae* also assigns *F. capillipes* Gagnep. from mainland Asia and the Madagascan *F. assimilis* Baker and *F. ampana* C.C.Berg to this group; these have not yet been included in phylogenetic studies. The *Albipilae* clade can be distinguished from the *Oreosycea* clade primarily by the presence of hairs on the inner surface of the fig receptacle. The exact circumscription of the *Albipilae* clade awaits comprehensive species sampling.

Caricae

This clade (Fig. 3D; 100% BS/PP = 1.00) includes only the domesticated Mediterranean *F. carica* and *F. palmata* Roxb. extending from north-eastern Africa to Pakistan. Together with *F. iidaiana* Wilson from Bonin Island (Japan), these three species formerly constituted *Ficus* section *Ficus* subsection *Ficus* Berg & Corner, but *F. iidaiana* is a member of *Frutescentiae* in the present study. The traditional subgenus *Ficus* is polyphyletic consisting of three strongly supported major clades, *Caricae*, *Eriosycea* and *Frutescentiae*, corresponding to clear-cut subdivisions by Berg & Corner (2005; Berg, 2003c). The relationship of the *Caricae* clade is uncertain. *Ficus carica* is the type of genus *Ficus*.

Mixtiflores

This clade (Fig. 3D; 100% BS/PP = 1.00) corresponds to subgenus *Urostigma* (Gasp.) Miq. excluding section *Urostigma* (Gasp.) Miq and includes *c*. 265 monoecious species in two subclades, one (100% BS/ PP = 1.00) consisting of section *Conosycea* Corner (98% BS/PP = 0.99) and (sub)section *Malvanthera* Corner (100% BS/PP = 0.99), and the other (100% BS/PP = 1.00) including section *Galoglychia* (Gasp.) Endl. (66% BS/PP = 0.68) and section *Americanae* Miq. (100% BS/PP = 1.00). In all the species, the staminate flowers are scattered among the pistillate flowers in the fig cavity.

Galoglychia

This clade (Fig. 3E; 66% BS/PP = 0.68) corresponds to the African section *Galoglychia* (Gasp.) Endl. Early studies (Rønsted et al., 2005, 2007, 2008a) suggested that *Galoglychia* is paraphyletic to *Americanae*, but monophyly of *Galoglychia* has been confirmed by later studies (Renoult *et al.*, 2009; Cruaud *et al.*, 2012b). Detailed phylogenetic studies of section *Galoglychia* were published by Rønsted *et al.* (2008b) and Renoult *et al.* (2009). Based on nuclear sequences, Rønsted, Salvo & Savolainen (2007) found that *Galoglychia* consists of two major clades in Africa, possibly corresponding to two main centres of diversity. One clade comprises members of subsections *Platyphylla*e (Mildbraed & Burret) C.C.Berg and *Chlamydodorae* (Mildbraed & Burret) C.C.Berg, are more concentrated in East Africa, and extend to Madagascar and neighbouring archipelagos (Comoros, Mascarenes, Aldabra Islands and Seychelles) and is sister to *Americanae* in the study by Rønsted *et al.* (2007). The other main clade (includes members of subsections *Caulocarpae* (Mildbraed & Burret) C.C.Berg, *Cyathistipulae* (Mildbraed & Burret) C.C.Berg, *Crassicostae* (Mildbraed & Burret) C.C.Berg and *Galoglychia*, which are concentrated in West and Central Africa (Berg, 1986). Renoult *et al.* (2009) found discordance of highly variable plastid data with the nuclear data, possibly caused by introgressive hybridization. In the present study, the six subclades are evident, but their relationships are not well supported.

Americanae

This clade (Fig. 3E; 100% BS/PP = 1.00) corresponds to Neotropical section *Americanae* Miq. including *c*. 110 species of hemi-epiphytes with low sequence variation possibly representing a rapid radiation. A detailed study of the Americanae clade has been published by Machado *et al.* (2018).

Conosycea

This clade (Fig. 3F; 99% BS/PP = 0.99) corresponds to section *Conosycea* (Miq.) Corner (Corners, 1965) plus Corner's acceptance of section *Stilpnophyllum* Endl. (*Ficus elastica* Roxb.) and section *Leucogyne* (*F. amplissima* Sm. and *F. rumphii* Bl.), which Berg & Corner (2005) considered members of section *Urostigma s.s.* (= subsection *Urostigma*). A number of clades are resolved in section *Conosycea*, some of which correspond to traditional series and subseries, but the subdivisions proposed by Corner (1965) and Berg and Corner (2005) are not reflected.

Malvanthera

This clade (Fig. 3F; 98% BS/PP = 0.99) corresponds to section *Malvanthera* Corner, which was reduced to subsection rank by Berg & Corner (2005). The *Malvanthera* clade includes 23 Australasian species with centres of diversity in New Guinea and Australia. The section was included in section *Stilpnophyllum* Endl. by Berg & Corner (2005) together with *F. elastica*, but phylogenetic evidence shows that *F. elastica* is a member of the *Conosycea* clade and section *Stilpnophyllum sensu* Berg & Corner (2005) is therefore polyphyletic. A detailed phylogenetic tree of the *Malvanthera* clade was published by Rønsted *et al.* (2008b) and relationships in that study are mirrored in the present study including the same sampling for the section. Rønsted *et al.* (2008b) also highlighted problems with the species concept of Berg & Corner (2005) for *Malvanthera*. In particular Berg & Corner (2005) united the majority of the New Guinea species under *F. hesperidiiformis* King, which is not supported by phylogenetic evidence (Rønsted *et al.*, 2008b), and at the same time Berg & Corner (2005) kept a narrow species concept for the Australian species.

Pharmacosycea

This clade (Fig. 3D; 100% BS/PP = 1.00) corresponds to section *Pharmacosycea* (Miq.) Benth. & Hook., includes *c.* 25 species restricted to the Neotropics and was recovered as sister to all other *Ficus* spp. Polyphyly of subgenus *Pharmacosycea* has been firmly established in molecular phylogenetic trees (e.g. Weiblen, 2000; Rønsted et al., 2005, 2008a; Cruaud *et al.*, 2012b). Morphologically, the *Pharmacosycea* clade is similar to the Old World section *Oreosycea s.s.*, the remaining section of subgenus *Pharmacosycea* (*sensu* Berg & Corner, 2005). However, former subgenus *Pharmacosycea* is polyphyletic and all three sections of this subgenus (Oreosycea, Albipilae, Pharmacosycea; Fig. 2) should be recognized as independently evolving lineages. Relationships in section *Pharmacosycea* were recently evaluated by Pederneiras, Romaniuc-neto & Mansano (2015), although species names were not fully clarified.

Taxonomic implications

A formal revision of *Ficus* awaits additional taxon sampling, but it is our hope that this comprehensive view of the phylogenetics of *Ficus* and recognition of well-supported clades will allow researchers to more easily discuss and describe the evolution and diversity of figs by making use of these informal clade names. In particular, we would advocate that further revision of Moraceae would formally recognize Involucrata either as a clade in a rank-free taxonomy or at the appropriate rank in a rank-based classification system, as many key evolutionary events happened along this branch. For *Ficus*, we strongly recommend abandoning the names associated with non-monophyletic subgenera of figs and instead use the proposed clade names until further taxonomic revision. In Castilleae, we reinstate the genus *Noyera* based on the molecular phylogenetic evidence presented in this paper.

Noyera Trécul, Ann. Sci. Nat. Bot. III. 8: 135. 1847. Type species: *Noyera rubra* Trécul.

Perebea section *Noyera* (Trécul) Engl., *in* Engler & Prantl, Nat. Pflanzenfam. 3(1): 84. 1889.

Conclusions

Despite the extensive study of *Ficus* due to its striking diversity and brood-site pollination mutualism, the deep evolutionary history of the group cannot be understood without attention to and comparison with its closest relatives, Castilleae. We introduce the clade Involucrata to recognize that *Ficus* and Castilleae comprise a group united by a trait that is central to their inflorescence morphology and pollination syndromes, the involucral bracts. Here, with the first intensive sampling of Castilleae and the most comprehensive phylogenetic reconstruction of *Ficus* to date, we delineate and name clades that are well supported to guide sampling in future studies of Involucrata and highlight those aspects of phylogenetic tree that warrant further investigation.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supplementary Table S1. Voucher information for Involucrata.

Supplementary Table S2. Amplification and sequencing primers used with ITS, *G3pdh*, and GBSSI.